PRODUCING LENTIVIRUS IN 293FT CELLS TRANSFECTION PROTOCOL

This protocol is adapted from Invitrogen's ViraPower™ Lentiviral Expression System by the Gene Expression Lab.
This protocol is for use with ViraPower™ Lentiviral Expression System. For additional technical inquiries, contact Technical Service at (800) 955-6288 *or www.invitrogen.com*

Recommendation:

- Follow the procedure below to co-transfect 293FT cells. We recommend including a negative control (no DNA, no Lipofectamine™ 2000) in your experiment to help you evaluate your results. You will need 6 x 106 293FT cells for each sample.

BEFORE STARTING THE EXPERIMENT

- The day before transfection, plate 6 x 106 293FT cells in a 100 cm tissue culture plate such that they will be 70-80% confluent on the day of transfection
- On the day of transfection, remove the culture medium from the 293FT cells and replace with 10 mL of growth medium (or Opti-MEM® I Medium) containing serum. Do not include antibiotics in the medium.
- Warm Opti-MEM I Medium without Serum AND with Serum to RT
- Thaw Plasmid DNA on ice
- Mix Lipofectamine 2000 well by vortexing
- Clean the rotor for virus concentration.

Transfection Procedure

1. **For each transfection sample**, prepare DNA-Lipofectamine™ 2000 complexes as follows:

i. In a sterile 5 mL tube, dilute 9 µg of the ViraPower™ Packaging Mix and 3 µg of pLenti expression plasmid DNA (12 µg total) in 1.5 mL of Opti-MEM® I Medium without serum. Mix gently.
ii. In a separate sterile 5 mL tube, mix Lipofectamine™ 2000 gently before use, then dilute 40:l in 1.5 mL of Opti-MEM® I Medium without serum. Mix gently and incubate for 5 minutes at room temperature.
iii. After the 5 minute incubation, combine the diluted DNA with the diluted Lipofectamine™ 2000. Mix gently.
iv. Incubate for 20 minutes at room temperature to allow the DNA-Lipofectamine™ 2000 complexes to form. The solution may appear cloudy, but this will not impede the transfection.
v. Add the DNA-Lipofectamine™ 2000 complexes drop wise to each plate of cells. Mix gently by rocking the plate back and forth. Incubate the cells overnight at37°C in a CO2 incubator.

2. The next day, Remove the medium containing the DNA-Lipofectamine™ 2000 complexes from incubation and replace with complete culture medium containing sodium pyruvate (*i.e.* D-MEM containing 10% FBS, 2 mM L-glutamine, 0.1 mM MEM Non-Essential Amino Acids, 1% penicillin/streptomycin).

Note: Expression of the VSV G glycoprotein causes 293FT cells to fuse, resulting in the appearance of multinucleated syncitia. This morphological change is normal and does not affect production of the lentivirus.

3. Harvest virus-containing supernatants 72 hours post transfection by removing medium to a 15 mL sterile, capped conical tube.

Caution: **Remember that you are working with infectious virus at this stage. Follow the recommended guidelines for working with BL-2 organisms**.

4. Centrifuge at 3000 rpm for 15 minutes at +4°C to pellet cell debris.
5. Filter the viral supernatant through a syerile, 0.45µm low protein binding filter after the low speed centrifugation to remove the cellular debris, using on Millex-HV 0.45µm PVDF filters from Millipore Cat # SLHVR25LS.
6. Pipet viral supernatants into cryovials in 1 mL aliquots. Store viral stocks at-80°C. Proceed to **Titering** by Blasticidin or Zeocin selection.

Concentration of Lentivirus using Millipore Centricon Filter Method

1. Harvest virus containing supernatants 72 hours post transfection by removing medium to a 15-50 mL sterile, capped conical tube.
2. Centrifuge at 3000rpm for 20 minutes at 4°C to pellet cell debris.

3. Filter the supernatant through a sterile 0.45um low protein-binding filter after the low speed centrifugation to remove any remaining cellular debris (Millex-HV 0.45uM PVDF filters from MILLIPORE).
4. Transfer the supernatant to a Centricon plus –20 filters (Millipore; Cat # UFC2BHK08) and centrifuge at 2,500rpm for 15 minutes at 4°C for 13 mL of supernatant (Time is dependent on the amount of supernatant).
5. Collect the sample by inverting the filter into the sterile cup provided and spin at 1,000rpm for 2-3 minutes.
6. Aliquot 25-50 µL/tube, store at -70°C.